Description of *Caldalkalibacillus uzonensis* sp. nov. and emended description of the genus *Caldalkalibacillus*

Weidong Zhao,^{1,2} Chuanlun L. Zhang,^{1,2} Christopher S. Romanek^{2,3} and Juergen Wiegel⁴

Correspondence
Juergen Wiegel
jwiegel@uga.edu

¹Department of Marine Sciences, University of Georgia, Athens, GA 30602, USA

²Savannah River Ecology Laboratory, University of Georgia, Aiken, SC 29802, USA

³Department of Geology, University of Georgia, Athens, GA 30602, USA

⁴Department of Microbiology, University of Georgia, Athens, GA 30602, USA

Strain JW/WZ-YB58^T, a thermophilic (42–64 °C), aerobic, alkalitolerant (pH^{25 °C} 6.4–9.7), heterotrophic, sporulating, retarded-peritrichously flagellated and slightly curved rod-shaped bacterium, was isolated from the hot spring Zarvarzin II in the East Thermal Field of the Uzon Caldera, Kamchatka (Far East Russia). The isolate tolerated high concentrations of CO. The major membrane phospholipid fatty acids of JW/WZ-YB58^T included iso-C_{15:0} (24.5%), anteiso-C_{15:0} (18.3%) and iso-C_{17:0} (17.5%). The G + C content of the genomic DNA is 45 mol% (HPLC method). Based on 16S rRNA gene sequence analysis and physiological properties, isolate JW/WZ-YB58^T (=ATCC BAA-1258^T =DSM 17740^T) is proposed as the type strain of *Caldalkalibacillus uzonensis* sp. nov. In contrast to the type species *Caldalkalibacillus thermarum*, a catalase-reaction-positive aerobe, *C. uzonensis* was catalase-reaction-negative; thus the description of the genus *Caldalkalibacillus* is emended to include a catalase-reaction-negative species.

An aerobic heterotrophic, flagellated and slightly curved rod-shaped bacterium, strain JW/WZ-YB58^T, which tolerated high concentrations of CO (up to 90%) in the head space, was isolated from a hot spring located in the Uzon Caldera (Kamchatka, Far East Russia) and was named '*Thermalkalibacillus uzonensis*' (Zhao *et al.*, 2006). Analysis of 16S rRNA gene sequences suggested that JW/WZ-YB58^T instead represents a novel species of the genus *Caldalkalibacillus* (Xue *et al.*, 2006), which previously contained only the type strain of the type species, *Caldalkalibacillus thermarum* HA6^T, an aerobic, heterotrophic, thermophilic bacterium (Xue *et al.*, 2006). The 16S rRNA gene sequence of *C. thermarum* HA6^T was 96.5% similar to that of strain JW/WZ-YB58^T and 99% similar to that of *Bacillus* sp. TA2.A1 (Peddie *et al.*, 2000), suggesting that strain JW/WZ-YB58^T represents a separate species of the genus *Caldalkalibacillus*. The next closest neighbour, *Bacillus horti*, showed 92–93% 16S rRNA gene sequence similarity to *Caldalkalibacillus* strains (Fig. 1).

Physiological and biochemical properties of strain JW/WZ-YB58^T were described in detail by Zhao *et al.* (2006). Major

phospholipid fatty acids of strain JW/WZ-YB58^T included iso-C_{15:0} (24.5%), anteiso-C_{15:0} (18.3%), iso-C_{16:0} (9.9%), iso-C_{17:0} (17.5%), anteiso-C_{17:0} (9.7%) and C_{16:0} (7.6%). Strain JW/WZ-YB58^T and *C. thermarum* HA6^T shared many properties. For example, both were strictly aerobic, thermophilic, alkalitolerant, Gram-staining-positive, sporulating rods that were able to grow on a variety of organic substrates including yeast extract, carbohydrates and organic acids. Both species contained iso-C_{15:0} (24.5–33.8%) and iso-C_{17:0} (17.5–35.5%) as major phospholipid fatty acids and the G + C contents of genomic DNA of both strains are about 45 mol% (although one was determined using the HPLC method and the other by *T_m*).

However, the two species differed significantly in several properties (Table 1). For example, in contrast to the type species of the genus *C. thermarum*, strain JW/WZ-YB58^T was motile, contained two to five peritrichously inserted flagella, and was unable to utilize alcohols and sugar alcohols (e.g. D-sorbitol, D-mannitol, glycerol) as carbon and energy sources. Strain JW/WZ-YB58^T also contained anteiso-C_{15:0} as the second most abundant fatty acid and was catalase-reaction-negative (Table 1). Strain JW/WZ-YB58^T is therefore assigned to a novel species of the genus *Caldalkalibacillus*, *Caldalkalibacillus uzonensis* sp. nov., and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JW/WZ-YB58^T is DQ221694.

Fig. 1. Phylogenetic tree based on 1448 bp 16S rRNA gene sequences. Relationships between sequences of strain JW/WZ-YB58^T (*Caldalkalibacillus uzonensis* sp. nov.) and other related, mainly alkaliphilic and thermophilic strains were calculated using the neighbour-joining method with MEGA 3.1 software. Bootstrap analysis of 1000 replicates was performed and values greater than 50% are shown at nodes. Bar, 0.01 changes per nucleotide.

the last difference described above necessitates the emendation of the genus description.

Emended description of the genus *Caldalkalibacillus* Xue *et al.* 2006

The description is based on that provided by Xue *et al.* (2006), with the following amendments. The genus contains catalase-reaction-positive and catalase-reaction-negative species. Predominant cellular phospholipid fatty acids include iso-C_{15:0}, anteiso-C_{15:0} and iso-C_{17:0}.

Description of *Caldalkalibacillus uzonensis* sp. nov.

Caldalkalibacillus uzonensis (u.zo.nen'sis. N.L. masc. adj. *uzonensis* pertaining to the isolation habitat of the type strain, the Uzon Caldera, east of Mt Uzon in Kamchatka, in Far East Russia).

The description is based on the previous detailed publication as '*Thermalkalibacillus uzonensis*' (Zhao *et al.*, 2006). Cells are Gram-type- (Wiegel, 1981) and Gram-staining-positive, straight to slightly curved rods, 0.7–0.8 × 5.5–12 µm. Terminally located spherical spores (1.2–1.6 µm in diameter) cause swelling of the mother cell. Cells are motile with two to five peritrichously inserted flagella. Growth is observed only under aerobic conditions. Colonies on nutrient broth agar are circular with entire edges and transparent. Swarming occurs when colonies age. Oxidase reaction, ONPG hydrolysis and gelatin hydrolysis are positive. Catalase reaction, Voges–Proskauer reaction, indole production, urea hydrolysis and starch hydrolysis are negative. At pH^{25 °C} 8.0, the growth temperature range is 42–64 °C, with optimum growth at 50–52 °C. The pH^{25 °C} range is 6.4–9.7, with optimum growth at pH^{25 °C} 8.2–8.4 when grown at 52 °C. NaCl is tolerated at 0–6% (w/v). Cells grow on complex substrates (such as yeast extract), carbohydrates and acids but not on alcohols or sugar alcohols. Acid production occurs from trehalose, lactose, sucrose, arabinose and fructose. Major phospholipid fatty acids are iso-C_{15:0} (24.5%), anteiso-C_{15:0} (18.3%), iso-C_{16:0} (9.9%), iso-C_{17:0} (17.5%), anteiso-C_{17:0} (9.7%), and C_{16:0} (7.6%). The G+C content of genomic DNA of the type strain is 45 mol% (HPLC; Mesbah *et al.*, 1989). Cells do not grow

Table 1. Differentiating characteristics of strain JW/WZ-YB58^T and related species of the *Firmicutes*

Data for reference species were taken from Xue *et al.* (2006) (*Caldalkalibacillus thermarum*) and Yumoto *et al.* (1998) (*Bacillus horti*). +, Positive; w, weak; –, negative; s, spherical; e, ellipsoidal; T, terminal; ST, subterminal. None of the taxa grows at 10 °C, and all three grow at pH 9.

Characteristic	JW/WZ-YB58 ^T	<i>C. thermarum</i>	<i>B. horti</i>
Spore properties			
Shape	S	S	E
Position	T	T	ST
Catalase	–	+	+
Growth at/in:			
40 °C	–	–	+
50 °C	+	+	–
pH 7	+	–	+
10% (w/v) NaCl	–	–	+
Hydrolysis of starch	–	w	+
Reduction of nitrate	–	–	+
Major phospholipid fatty acids	iso-C _{15:0} , anteiso-C _{15:0} , iso-C _{17:0}	iso-C _{15:0} , iso-C _{17:0}	iso-C _{15:0} , anteiso-C _{15:0}
DNA G+C content (mol%)	45	45.2	40–41

chemolithoautotrophically on 1–5 % v/v CO or 5 % CO₂ with or without H₂ and do not oxidize CO, even at low concentrations (100 p.p.m.). Cells growing under shaking tolerate up to 90 % (v/v) CO in the air-balanced headspace.

The type strain is JW/WZ-YB58^T (=ATCC BAA-1258^T =DSM 17740^T), which was isolated from a microbial mat sample collected from the edges of the hot spring Zarvarzin II in the East Thermal Field of the Uzon Caldera, Kamchatka (Far East Russia).

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